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Simultaneous estimation of Zidovudine and Lamivudine tablets by RP-HPLC method

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Abstract: A high-performance liquid chromatographic method was developed and validated for the determination of two antiretroviral drugs viz. Zidovudine and Lamivudine in combined pharmaceutical tablets. The different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined. Chromatography was carried out on a reversed-phase C-18 Phenomenex column with mobile phase Water: Acetonitrile (60:40) at 1.0 ml/min with pH 3.5 and detection wavelength 272 nm. The linearity of the calibration curves for each analyte in the desired concentration range is good (r^2> 0.999). The percentage recovery of the zidovudine and lamivudine were found to be 99.10% and 97.2% respectively. Similarly the RSD value for precision was also found to be within the acceptable limit. The proposed methods are highly sensitive, precise and accurate and hence were successfully applied for the reliable quantification of API content in the commercial formulations of Zidovudine and Lamivudine.

Keywords: Zidovudine, Lamivudine, RP-HPLC, Mobile phase.

Introduction:

One of the deadliest and unmanageable chronic health catastrophes is HIV/AIDS. Fixed dose combinations (FDCs) form the main stay in clinical management of HIV-1 infection as they offer several advantages over single products with respect to storage, prescribing, dispensing, patient use, consumption and disease management[1]. Combination of the drugs into fixed dose combinations (FDCs) has been an essential constituent of the Highly Active Anti-retroviral (HAART) therapy[2].

Lamivudine (Fig-1a) (L-2', 3'-dideoxy-3'-thiacytidine) is a pyrimidine analog and Zidovudine (Fig-1b) is a nucleoside analog reverse transcriptase inhibitor both active against HIV-1 and HIV-2. Lamivudine is a nucleoside analog having potent in vitro and in vivo inhibitory activity against HIV reverse transcriptase. Lamivudine specifically refers to the (−) enantiomer of the cis racemate and is marketed as tablets in different strengths. It is rapidly absorbed with a bioavailability of approximately 80%. It is synergistic with other antiretroviral agents including stavudine, zidovudine, didanosine, nevirapine and delavirdine [3-4].

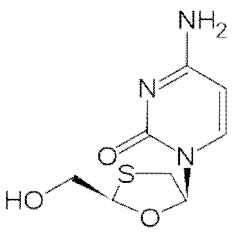

Fig.1(a). Lamivudine.

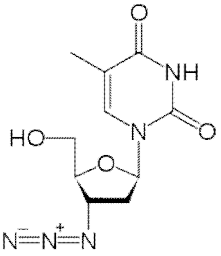

Fig.1(b). Zidovudine

Literature revealed that few methods have been reported for the individual estimation of Zidovudine and Lamivudine and in combination with other drugs [5-7]. The method developed is precise, simple and specific RP-HPLC method to determine Zudovudine and Lamivudine in pharmaceutical dosage forms.

Materials and Methods:

Materials:

Zidovudine and Lmaivudine pure standards were received as gift samples from Cipla Pharmaceuticals, Gujart (India). All other reagents used were HPLC grade.

Apparatus:

The HPLC system was waters HPLC consisted with the pump-alliance 2695 seperation module, column-phenomenex luna 5µ C18 (12) 100A,(250×4.6×i.d, 5µ), auto sampler, detector was waters 2996 and prominence diode array detector. Other instruments used were, Melter balance AY 220, Elico pH meter LI 127, Melter Ultrasonicatior and Millipore membrane filter. Shimadzu balance AY 220 and sonica ultrasonic cleaner were used

Pharmaceutical preparations:

Commercial FDC formulations from Cipla pharmaceuticals (COMBIVIR) were procured and were assayed. The declared content was as follows: Zidovudine 300mg and Lamivudine 150mg in a tablet or dosage forms.,

Methods:

Preparation of Standard solutions:

Primary stock solution concentration of Zidovudine and Lamivudine 1000 µg/ml was prepared. All measurements were made at room temperature. The standard solutions were prepared by proper dilutions of the primary stock solution with acetronitrile and water (20:80) to obtain working standards in the concentration range of 30-90 µg/ml of Zidovudine and 15-75µg/ml of Lamivudine.

Sample preparation:

The tablet containing 300 mg Zidovudine and 150 mg Lamivudine were weighed, powdered and dissolved in acetronitrile and water (20:80) made to 1000 µg/ml. The stock solution of zidovudine and Lamivudine was suitably diluted to give the mixture concentration of 30 & 15, 45 & 30, 60 & 45, 75 & 60 and 90 &75 µg/ml for measurement. The contents were mixed thoroughly and filtered through 0.45µ membrane filter and sonicated for 20 min.

Recording of chromatogram:

After various trials the following chromatographic conditions were finally optimized for the simultaneous estimation of Zidovudine and Lamivudine in a tablet dosage form. Mobile phase ratio of 60: 40 (Water: ACN), pH 3.5(adjusted with Orthophosphoric acid, wave length 272 nm flow rate1.0 ml/min, after a steady baseline the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas were found and finally 20 µg/ml of the standard solution of the individual samples of Zidovudine and Lamivudine and mixed standard solutions were injected and the chromatograms were recorded. The typical chromatograms of the standard solutions were recorded for the repeatability and the respective figure was given in Fig. 2.

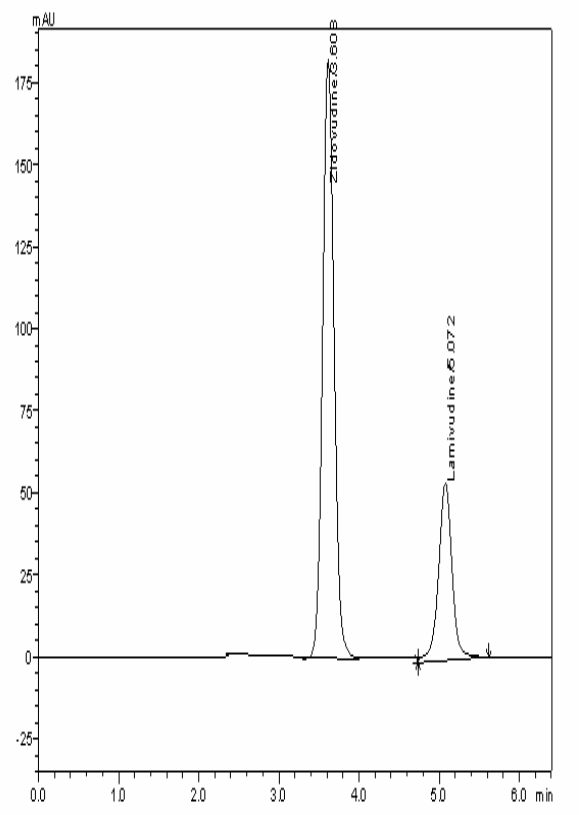

Fig-2: Chromatogram of Linearity Standard solution ofZidovudine 90 µg/ml and Lamivudine 75 µg/ml

The procedure was repeated using the sample solution and the chromatogram was shown in Fig.3. The peak areas were noted for the standard and sample solutions and compared. The elution order of mixture was found as Zidovudine (retention time 3.6 min) and Lamivudine (retention time 5.0 min).

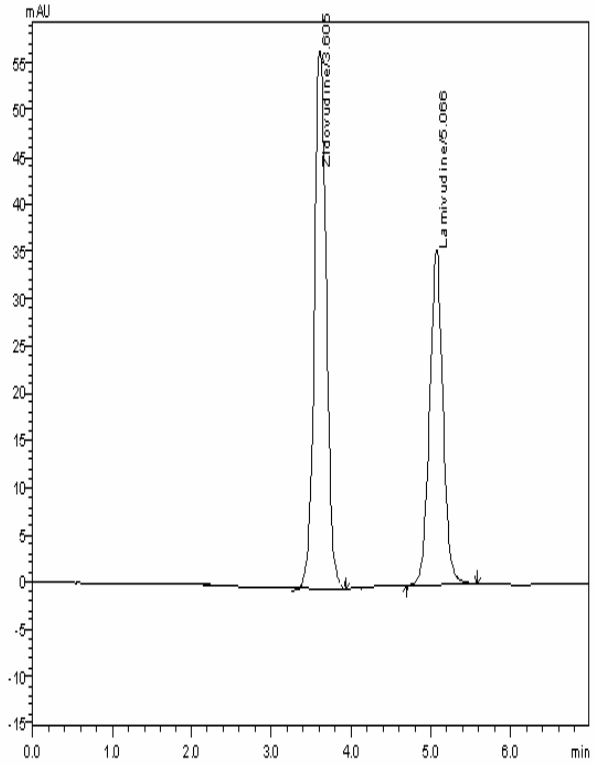

Fig 3: Chromatogram of sample solution

Result

The assay procedures was repeated for 6 times and mean peak area, mean peak ratio, mean weight of standard drugs, mean weight of sample taken for the assay were calculated.

The percentage of individual drugs were found in the formulations, mean and relative standard deviation in formulations were calculated and given in the Table 1. The peak area ratios of standards and samples solutions were calculated.

Validation of the method:

The different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ) and System suitability studies were determined and given in the tables (2-6).

Discussion:

RP-HPLC method:

The scope of the present work is to expand the optimization of the chromatographic conditions, to develop RP-HPLC method for the estimation of drugs in selected multi-component dosage forms. The developed method was also validated.

The linearity and range was found to be in the range of 30-90 µg/ml for Zidovudine and 15 – 75 µg/ml Lamivudine. The correlation coefficient of Zidovudine and Lamivudine were found to be 0.9996 and 0.9991 respectively (Fig 4 & 5), which indicates a perfect correlation. The developed method was validated for accuracy, precision, and system suitability. The percentage recovery of Zidovudine and Lamivudine were found to be 99.10 % and 97.2 % respectively.

Table 1: Analysis of Formulation

Drug	Lable claim (mg/tablet)	Estimated Amount (mg/tablet)	% Lable claim	% RSD n=6
Zidovudine	30	29.77	99.09	0.17
Lamivudine	15	14.420	97.12	0.59

Table 2: Accuracy (Recovery Studies)

Drug	**Amount added (mg/ml)**	**Amount recovered (mg/ml)**	**Recovery (%)**	**Average recovery ±SD (%) (n=6)**
Lamivudine	1.19 2.85 3.80	1.17 2.84 3.74	97.40 97.99 97.36	97.60 ± 0.351
Zidovudine	3.80 5.70 7.60	3.72 5.60 7.50	98.04 98.33 98.40	98.26 ± 0.190

Table 3: Intraday Studies

Area of Zidovudine	Mean	% RSD	Area of Lamivudine	Mean	% RSD n=6
7294830 7285075 7259111 7314205 7252800 7262799	7278137	0.14	3137848 3186560 3158735 3149222 3146927 3147836	3154522	0.54

Table 4: Inter day Studies

Day	Mean Rf of Zidovudine (% RSD)	Mean Rf of Lamivudine (% RSD)
Day 1	7278137 (0.14)	3154522 (0.54)
Day 2	7272106 (0.17)	3145596 (0.41)
Day 3	7265578 (0.16)	3150167 (0.43)

Table 5: Linearity range of Zidovudine and Lamivudine:

Zidovudine		Lamivudine	
Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
30	4035363	15	2559975
45	5867889	30	2870987
60	7465929	45	3176991
75	9207462	60	3468287
90	10983727	75	3871462

Table 6: system suitability studies

Parameters	Zidovudine	Lamivudine
Theoretical plates / meter	6935	5991
Resolution	-	2.45
Capacity factor	0.15	0.19
Tailing factor	0.65	1.76
LOD (µg /ml)	1.06	3.11
LOQ (µg /ml)	3.40	9.42

The RP-HPLC method was developed for the simultaneous estimation of Zidovudine and Lamivudine in combined dosage forms which can be conveniently employed for routine quality control in pharmaceutical dosage forms.

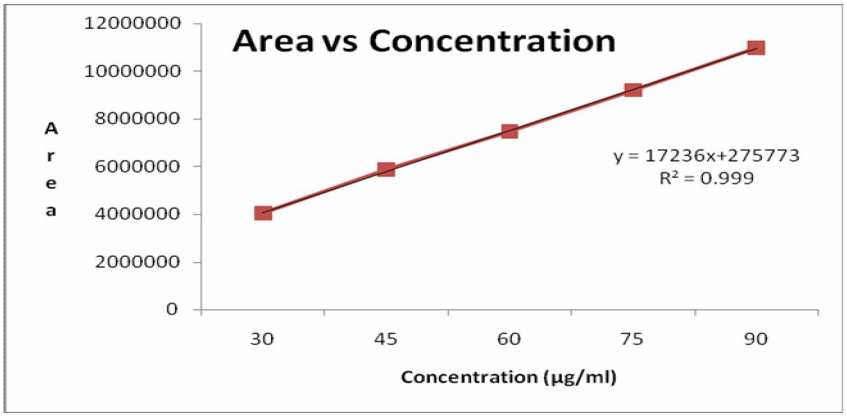

Fig 4: Linearity curve of ZIDOVUDINE

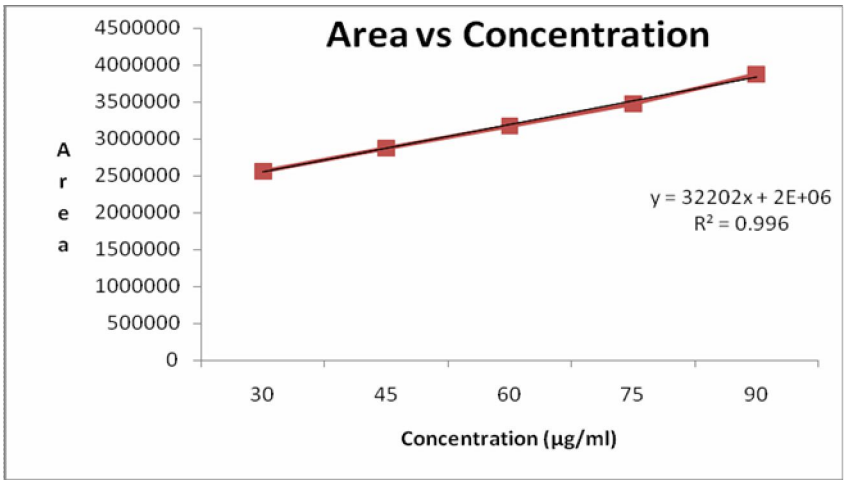

Fig. 5: Calibration curve of LAMIVUDINE

Acknowledgements

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